

Growth Performance, Semen Quality Characteristics and Hormonal Profile of Male Rabbit Bucks Fed *Rubia cordifolia* Root Extracts

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ABSTRACT

32 – 7 weeks weaned male rabbits (Newzealand × Chinchilla) weighing 611.3 ± 10 g were randomly distributed into 4 groups of 8 rabbits per treatment with one animal per replicate in a completely randomized design. Rabbits in treatment 1 (control) designated as T1 was fed basal diet with 0 mL *Rubia cordifolia* root extracts (RCE) while T2, T3 and T4 were fed basal diet with 20 mL, 40 mL and 60 mL per litre of water/day. Basal diet was formulated to meet the nutrient requirements of growing rabbits according to the recommendation of National Research Council (NRC, 1977). The experiment lasted for 12 weeks during which strict biosecurity measures were observed. Feed and water were also given *ad libitum*. Gas chromatography mass spectrometry of *Rubia cordifolia* root extracts reveals the presence of 21 bioactive compounds which accounts for 92.46 %. 9-Octadecenoic acid had the highest concentration (29.16 %) while 4-Methoxy-2-nitroformanilide had the lowest concentration (0.02 %). Average body weight gain (ADWG) and feed conversion ratio of rabbits in T4 were better ($P < 0.05$) compared to the other treatments. Average daily feed intake (ADFI) in T1 was similar to T2 and T3 but slightly higher than T4 ($P > 0.05$). Highest mortality was recorded in T1 (2.51 %) followed by T2 (0.05 %) none was recorded in T3 and T4 ($P < 0.05$). Testosterone, luteinizing hormone and thyroid stimulating hormone values were significantly ($P < 0.05$) influenced by the treatments while follicle stimulating hormone were not significantly ($P > 0.05$) different among the treatments. Semen results showed a significant ($P < 0.05$) decrease in sperm concentration, live sperm and motility with a significant ($P < 0.05$) increase in abnormal sperm compared to the other treatments. It can be concluded that *Rubia cordifolia* root extracts has bioactive compounds with therapeutic properties and could be tolerated by rabbit bucks up to 60 mL per litre without causing any negative effect on the general health and performance of animals.

Keywords: *Rubia cordifolia* root extracts, performance, phytochemicals, hormones, semen

1. INTRODUCTION

Plant derived additives have been identified to contain bioactive compounds with effects similar to antibiotic growth promoters in three main areas, i.e., gut micro-flora, antioxidant properties, and liver function without compromising intestinal health and/or the bird's genetic potential (Hernandez, *et al.*, 2004; Singh *et al.*, 2021). The addition of plants and their extracts into diets is aimed at improving the productivity of livestock through amelioration of undesired feed properties, promotion of the animal's production performance and improving the quality of food derived from those animals (Kolodziej-Skalska *et al.*, 2011; Agubosi *et al.*, 2022; Shittu *et al.*, 2021). Herbs such as herbs, spices, and various plant extracts have received increased attention as possible antibiotic growth promoters (Kim *et al.*, 2009; Alagbe and Ushie, 2022; Oluwafemi *et al.*, 2021).

Phytochemicals come from natural sources and are generally recognised as safe (GRAS) which make them good candidates to be used as feed additives in livestock production in comparison with antibiotics (Windisch *et al.*, 2008; Alagbe, 2017). The biological mechanism of action of phytochemicals depends on their chemical structure (Kim *et al.*, 2010; Alagbe *et al.*, 2022). Phytochemicals used as poultry feed additives can improve animal's health and performance because of their anti-microbial, anti-bacterial, anti-helminthic and anti-stress (Peric *et al.*, 2009; Shittu *et al.*, 2022) and anti-oxidant properties (Applegate *et al.*, 2010; Alagbe *et al.*, 2021), and their ability to modulate gut microbiota (Donoghue, 2009; Alagbe *et al.*, 2021) and enhance immune responses (Kim *et al.*, 2008; Adewale *et al.*, 2021). Environment factors such as nutrition, temperature, humidity, seasonal changes and animal management (health and housing) have a measurable effect on semen quality (Cross, 1998). Sperm morphology, motility, sperm concentration and volume per ejaculate are common criteria for evaluating semen quality (Ademola, 2003).

Rubia cordifolia also known as Indian madder belongs to the family Rubiaceae is a perennial flowering plant widely distributed in Asia (Bhatt and Kushwah, 2013). The stem, root and leaves of *Rubia cordifolia* is traditionally used for the treatment of gastrointestinal disorder, skin disease, malaria, typhoid, diabetes, liver diseases, menstrual and urinary diseases, cancer, inflammations, pneumonia, cough, chronic bronchitis, hemoptysis and other viral infections (Bhat *et al.*, 2018; Prajapati and Parmar, 2011; Musa *et al.*, 2020; Alagbe, 2017).

Several studies have demonstrated the anti-bacterial, antimicrobial, hepato-protective, antiviral, anti-rheumatic, immunomodulatory, cytotoxic, anti-ulcer, anti-fibrotic, antioxidant, anti-proliferative, antifungal and anti-helminthic properties in *Rubia cordifolia* extract (Boldizs *et al.*, 2006; Kalyoncu *et al.*, 2006; Gao *et al.*, 2003; Alagbe, 2020). Aqueous *Rubia cordifolia* root, leaf and stem bark extract have proven to suppress the activities of some pathogenic bacteria such as: *Salmonella spp*, *Staphylococcus pyogenes*, *Staphylococcus aureus*, *Bacteriodes spp* and *E. coli* in animals (Ino *et al.*, 1995; Kamuhabwa *et al.*, 2000; Wei *et al.*, 2011).

In view of these abundant potentials in *Rubia cordifolia*, this experiment was designed examine the growth performance, semen quality characteristics and hormonal profile of male rabbit bucks fed *Rubia cordifolia* root extracts.

2. MATERIALS AND METHODS

Site of the experiment

The experiment was carried out at Division of Animal Nutrition, Sumitra Research Institute, Gujarat, India with a coastline of 1,600 Km, 23° 13'N 72°41'E.

Collection, preparation and gas chromatography mass spectrometry (GC-MS) of *Rubia cordifolia* root extract (RCE)

Rubia cordifolia root was collected from Sumitra Research Institute in the month of April 2021. It was identified and authenticated by a certified taxonomist Dr. Xing Liu at the Department of Biological Sciences. Samples of *Rubia cordifolia* root were thoroughly washed with running tap water and air dried for 2 weeks to obtain a constant weight. Dried *Rubia cordifolia* root was pounded into powder with a mortar and pestle it was thereafter stored in a well labeled container.

Two hundred grams of dried *Rubia cordifolia* root powder was soaked in 1 liter for 72 hours, kept in the refrigerator at 4°C and stirred at 3 times daily using a spatula. The sample was filtered via a Whatman's No.1 filter paper (10 cm). Thereafter, the filtrate was stored in a laboratory labeled container and sent to the laboratory for further analysis.

Gas chromatography mass spectrometry (GC-MS) analysis of *Rubia cordifolia* root extracts (RCE) was carried out with Varian 450 GC system (Model 1100 series, China) with temperature and pressure range of 50°C to 450 °C isothermal 1079 PTV injector and 0 to 100 psi, consisting of splitless injector with total flow of 500 mL/minutes at 10 psi. The introduced sample (RCE) was passed through a rapid column at a cool down rate of 40 °C to 50 °C at 4.5 minutes with an electron range of 150eV. Bioactive compounds were identified with standard compounds in National Institute of Standard and Technology (NIST).

Animal management, diet formulation and experimental set-up

32 – 7 weeks weaned male rabbits (Newzealand × Chinchilla) weighing 611.3 ± 10 g were purchased from a reputable commercial breeding farms in Gujarat india and housed individually in an all wired galvanized cage measuring 50 cm × 50 cm × 30 cm: length × width × height suspended 120 cm above the ground, equipped with automatic nipple drinker and a metallic manual clay feeder was kept in each cage. Before the arrival of the animals, cages and pens were properly disinfected with Cid 2000 at 10 mL per 20 liters of water. On arrival, rabbits were given anti-stress and randomly distributed into 4 groups of 8 rabbits per treatment with one animal per replicate in a completely randomized design. Rabbits were acclimatized before the commencement of the experiment during which they were given prophylactic treatment against parasites (endo and ecto-parasites) with ivermectin injection and bacterial infections (Oxytrox®) strictly adhering to the manufacturers recommendation on drug administration.

Basal diet was formulated to meet the nutrient requirements of growing rabbits according to the recommendation of National Research Council (NRC, 1977) as presented in Table A. Rabbits in treatment 1 (T1) was fed basal diet with 0 mL *Rubia cordifolia* root extracts (RCE) while T2, T3 and T4 were fed basal diet with 20 mL, 40 mL and 60 mL per litre of water/day. The experiment lasted for 12 weeks during which strict biosecurity measures were observed. Feed and water were also given *ad libitum*.

Performance traits

Daily feed intake (g)

Daily feed intake was calculated by subtracting feed served from left over. It can be expressed as:

$$\text{Daily feed intake (g)} = \text{Feed served (g)} - \text{Feed left over (g)}$$

Body weight gain (g)

Body weight gain was calculated by subtracting final body weight from initial body weight

$$\text{Body weight gain (g)} = \text{Final body weight (g)} - \text{Initial weight gain (g)}$$

Feed conversion ratio (g)

Feed conversion ratio was calculated by dividing feed consumed by body weight gain as expressed below:

$$\text{Feed conversion ratio (g)} = \frac{\text{Feed consumed (g)}}{\text{Body weight gain (g)}}$$

Mortality was recorded as it occurs

Hormonal evaluation

Blood samples were collected from the marginal ear veins into vacutainer bottles without ethylene diamine tetra acetic acid from 4 randomly selected rabbits per treatment for hormonal assay using commercial diagnostic kit (AIA-360 Automated Immunoassay analyzer, USA) with dimension 1016 mm × 665 mm × 762 mm (width × depth × height).

Semen collection and evaluation

A 2-week period was used to train the bucks for semen collection. Semen was finally collected from the buck using the artificial vagina (AV) described by Herbert and Adejumo (1995). Prior to semen collection, the AV was warmed for a few minutes in warm water at a temperature slightly above body temperature and thereafter drained. Semen collection was done between 7.00 and 9.00 am to ensure that optimum quality semen were obtained.

The semen was promptly assessed for semen quality parameters such as semen colour, semen volume, mass activity, sperm motility, sperm concentration and percentage live sperm using Computer Assisted Semen Analyzer Bonraybio (Taichung City, Taiwan).

Statistical analysis

All data were subjected to one -way analysis of variance (ANOVA) using SPSS (23.0) and significant means were separated using Duncan's test of the same statistical package.

The model: $D_{ij} = \mu + \alpha_i + \beta_{ij}$ was used in this experiment:

Where D_{ij} = any of the response variables; i = the overall mean; α_i = effect of the x th treatment and β_{ij} = random error due to experimentation

Table 1: Chemical composition of basal diet

Ingredient	Quantity (Kg)
Maize	21.0
Wheat offal	35.0
Palm kernel meal	15.95
Groundnut cake	9.00
Soyabean meal	12.65
Bone meal	1.60
Limestone	1.00
Fish meal (65 %)	1.00
Salt	0.30
*Vitamins and minerals mixture	0.20
Rice offal	2.00
Methionine	0.10
Lysine	0.20
Total	100.0
Chemical analysis	(% DM)
Dry matter	89.31
Ash	6.60
Crude protein	15.21
Crude fibre	14.26
Ether extract	2.34
Nitrogen free extract	51.80
Digestible energy (Kcal/kg)	2584

*Each 1 kg contains: 10000 IU vitamin A, 2680 IU vitamin D₃, 10 IU vitamin E, 2.68 mg vitamin K, 10.68 mg calcium pantothenate, 0.022 mg vitamin B₁₂, 0.668 mg folic acid, 400 mg choline chloride, 26.68 mg chlorotetracycline, manganese 133.34 mg, 66.68 mg iron, 53.34 mg zinc, 3.2 mg copper, 1.86 mg iodine, 0.268 mg cobalt, 0.108 mg selenium.

3. RESULTS AND DISCUSSION

Bioactive compounds in *Rubia cordifolia* root extracts using GC-MS analysis

Medicinal plants contain several of bioactive compounds, such as phenolics, flavonoids, terpenoids, carotenoids, saponins and alkaloids in their stems, leaves, roots, seeds, flowers and twigs (Adewale et al., 2021). These compounds are widely used in the food, cosmetic and pharmaceutical industries because they possess; antioxidant (Shittu et al., 2020), antimicrobial (Singh et al., 2021), anti-inflammatory (Agubosi et al., 2022), hepato-protective (Olafadehan et al., 2021), antifungal and antiviral (Oluwafemi et al., 2020), immune-modulatory, cytotoxic, hypolipidemic, antibacterial, anti-tumor, antipyretic, antiparasitic, antifibrotic (Alagbe et al., 2022) and antiproliferative properties (Agubosi et al., 2022). Gas chromatography mass spectrometry of *Rubia cordifolia* root extracts reveals the presence of 21 bioactive compounds which accounts for 92.46 %. 3-deoxy-d-mannoic acid contains (0.44 %), 4-Methoxy-2-nitroformanilide (0.02 %), γ -terpinene (1.10 %), β -fenchol (0.40 %), 3-Allyl-6-methoxyphenol (1.67 %), Glycidol stearate (0.10 %), 2-Methyl -4-vinylphenol (2.05 %), α -cubebene (2.09 %), Carbonic acid (0.77 %), 9,12-Octadecanoic acid (25.06 %), α -longipinene (0.75 %), Terpinen-4-ol (1.04 %), 1,3 propanediol, 2-ethyl 2-hydroxymethyl (14.71 %), γ -terpinene (0.94 %), γ -eudesmol (1.13 %), 9-Octadecenoic acid (29.16 %), Torreyol- α -cadinol (0.07 %), 1,2-Cyclopentanedione (0.30 %), Ethylene diacrylate (0.50 %) and 4-methyl-2,3-hexadien -1-ol (11.20 %). 9-Octadecenoic acid had the highest concentration while 4-Methoxy-2-nitroformanilide had the lowest concentration. The result obtained in this study agrees with the findings of Mohammad et al. (2018); Kaur et al. (2008); Singh et al. (2021). A synergistic combination of these bioactive compounds allows *Rubia cordifolia* root extracts to be used in the treatment of various ailments due to their therapeutic properties (Bsau et al., 2005; Gupta et al., 2011); Raghad and Abdul (2017).

Table 2: Bioactive compounds in *Rubia cordifolia* root extracts using GC-MS analysis

Compounds	Area (%)	R.T (min)	Mole. wgt	Functions
3-deoxy-d-mannonic acid	0.44	10.78	162	Antimicrobial and antioxidant
4-Methoxy-2-nitroformanilide	0.02	13.09	196	Antifungal
γ -terpinene	1.10	9.43	188	Hepatoprotective and antifungal
β -fenchol	0.40	10.70	201	Anti-inflammatory, antibacterial and analgesics
3-Allyl-6-methoxyphenol	1.67	8.33	164	Antiprotozoal and cytotoxic
Glycidol stearate	0.10	10.56	340	Anti-androgenic, antiviral and anti-inflammatory
2-Methyl 4-vinylphenol	2.05	12.74	150	Hepato-protective, hypolipidemic, antimicrobial and antioxidant
α -cubebene	2.09	18.10	102	Antibacterial, antifungal, analgesics antipyretic and antioxidant
Carbonic acid	0.77	22.60	228	Antiviral and antioxidant
9,12-Octadecanoic acid	25.06	18.35	280	Cytotoxic, antioxidant, anti-inflammatory, antitumor, antifungal
α -longipinene	0.75	19.22	64	Anti-inflammatory, antioxidant, anti-depressant and antifungal
Terpinen-4-ol	1.04	14.30	87	Anti-fibrotic, anti-inflammatory and hypolipidemic
1,3 propanediol, 2-ethyl 2-hydroxymethyl	14.71	20.21	95	Antibacterial, anti-inflammatory, antipyretic, antihelminthic and antifungal
γ -terpinene	0.94	21.38	71	Antioxidant and anti-inflammatory
γ -eudesmol	1.13	30.93	48	Cytotoxic and hepato-protective
9-Octadecenoic acid	29.16	29.51	280	Antifungal
Torreyol- α -cadinol	0.07	17.25	52	Antiviral, hepato-protective and antioxidant
1,2-Cyclopentanedione	0.30	29.00	98	Anti-microbial, anti-proliferative, antiviral, antihelminthic and antibacterial
Ethylene diacrylate	0.50	14.20	170	Analgesics, antibacterial, antifungal
4-methyl-2,3-hexadien -1-ol	11.20	28.10	166	Antioxidant, anti-proliferative, antifungal and anti-inflammatory
Total	92.46			

R.T: reaction time (minutes)

Growth performance of rabbits fed different levels of *Rubia cordifolia* root extracts (RCE)

Growth performance of rabbits fed different levels of *Rubia cordifolia* root extracts (RCE) is presented in Table 3. Initial body weight (IBW), final body weight (FBW), weight gain (WG), average daily weight gain (ADWG), total feed intake (TFI), average daily feed intake (ADFI) and feed conversion ratio (FCR) ranged from 611.3 – 619.5 g, 1900.8 – 2300.4 g, 1282.1 – 1680.9 g, 8652.1 – 9103.1 g, 103.0 – 108.4 g, 4.00 – 4.84, 0.50 – 2.51 % respectively. ADWG value was highest in T4, intermediate in T2 and T3 and lowest in T1 ($P < 0.05$). Conversely, FCR value was maximum in T1, intermediate in T2 and T3 and lowest in T4 ($P < 0.05$). ADFI were not significantly ($P > 0.05$) affected among the treatments. Highest mortality rate was recorded in T1 (2.51 %) followed by T2 (0.50 %) while none were recorded in the other treatments ($P < 0.05$). Higher ADWG recorded among rabbits fed different levels of *Rubia cordifolia* root extract (RCE) indicates that the test ingredients enhanced nutrient utilization by stimulating the activities of enzymes and preventing dysbiosis due to the presence of several bioactive compounds (Table 2) thus enhancing performance (Sandip *et al.*, 2022; Muritala *et al.*, 2022). The result obtained in this study agrees with the findings of Ogbuwu *et al.* (2010); Oluwfemi and Alagbe (2019). The enhanced nutrient digestibility consequently enhances feed intake and health status of rabbits (Shittu *et al.*, 2021). RCE also possesses antioxidant and immune-modulatory properties due to the presence of α -cubebene, 3-deoxy-d-mannonic acid, Torreyol- α -cadinol, α -longipinene, 2-Methyl 4-vinylphenol and β -fenchol (Topala *et al.*, 2014; Kokila *et al.*, 2016).

Table 3: Growth performance of rabbits fed different levels of *Rubia cordifolia* root extract (RCE)

Parameters	Control (T1)	T2	T3	T4	SEM	LOS
IBW (g)	618.7	611.3	617.6	619.5	5.07	Ns
FBW (g)	1900.8 ^c	2022.8 ^b	2028.6 ^b	2300.4 ^a	20.80	*
WG (g)	1282.1 ^c	1411.5 ^b	1411.0 ^b	1680.9 ^a	9.33	*
ADWG (g/d)	15.26 ^c	16.80 ^b	16.80 ^b	20.01 ^a	0.90	*
TFI (g)	8652.1	8800.3	8872.1	9103.1	35.21	Ns
ADFI (g)	103.0	105.0	106.0	108.4	1.50	Ns
FCR	4.84 ^a	4.18 ^b	4.10 ^b	4.00 ^c	0.02	*
MOR (%)	2.51 ^a	0.50 ^b	-	-	0.001	*

Means in the same row not sharing same superscript are significantly ($P<0.05$) different

IBW: initial body weight; FBW: final body weight; WG: weight gain; ADWG: average daily weight gain; TFI: total feed intake; ADFI: Average daily feed intake; MOR: mortality; * Significant; Ns: non-significant; LOS: level of significant; T1: basal diet + 0 mL RCE; T2: basal diet + 20 mL RCE/day; basal diet + 40 mL RCE/day; basal diet + 60 mL RCE/day.

Hormonal profile of rabbits fed different levels of *Rubia cordifolia* root extracts (RCE)

Table 4 shows the hormonal profile of rabbits fed different levels of *Rubia cordifolia* root extracts (RCE). The hormones determined includes: testosterone (TES), follicle stimulating hormone (FSH), luteinizing hormone (LH) and thyroid stimulating hormone (TSH) which ranged from 2.06 – 4.00 (I.U/L), 6.50 – 6.98 (I.U/L), 8.80 – 12.30(I.U/L) and 0.93 – 2.03 (I.U/L) respectively. TES, LH and TSH values were significantly ($P<0.05$) influenced among the treatments. The values follow similar pattern and were highest in T4, intermediate in T2 and T3 and lowest in T1. This is a clear indication that the bioactive compounds in *Rubia cordifolia* root extract (RCE) is capable of activating the activities gonadotropin releasing hormone which stimulates the secretion of LH, which in turns stimulates gonadal secretion of testosterone, estrogen and progesterone. Conversely, FSH values were not significantly ($P>0.05$) different among the groups. The result obtained in this study agrees with the findings of Olatundun and Ogunlade (2020). According to Amao *et al.* (2013); Brucker *et al.* (1998), FSH and LH are secreted from the anterior pituitary cells of animals (gonadotrophs) with the aim of stimulating the gonads - in males, the testes and in females, the ovaries. Diminished secretion of LH or FSH can result in failure of gonadal function (hypogonadism), thus leading to poor sperm cell production (Ahemen *et al.*, 2013). FSH plays for sperm production. It supports the function of Sertoli cells, which in turn support many aspects of sperm cell maturation (Ghassabian and Trasande, 2018; Kay, 2014). Kilgour *et al.* (1984); Jiang (2007) reported that FSH is necessary for the establishment of the normal population of Sertoli cell and the stimulation of the production of androgen-binding protein from the Sertoli cells. Androgen-binding protein binds with the testosterone making it available for its function in spermatogenesis (Bearden and Fuquay, 1997; Lovecamp and Devis, 2003). Testosterone is responsible in maintaining optimum conditions for spermiogenesis, spermatozoa transport and semen deposition near the site of fertilisation in the female (Kay, 2014; Castellini, 2003).

Table 4: Hormonal profile of rabbits fed different levels of *Rubia cordifolia* root extracts (RCE)

Parameters	T1	T2	T3	T4	SEM	LOS
TES (I.U/L)	2.06 ^c	3.49 ^b	3.55 ^b	4.00 ^a	0.02	*
FSH (I.U/L)	6.50	6.78	6.93	6.98	0.06	Ns
LH (I.U/L)	8.80 ^c	10.10 ^b	12.09 ^a	12.30 ^a	0.15	*
TSH (I.U/L)	0.93 ^c	1.12 ^b	1.15 ^b	2.03 ^a	0.01	*

* Significant; Ns: non-significant; LOS: level of significant; T1: basal diet + 0 mL RCE; T2: basal diet + 20 mL RCE/day; basal diet + 40 mL RCE/day; basal diet + 60 mL RCE/day; TES: testosterone; FSH: follicle stimulating hormone; LH: luteinizing hormone; Thyroid stimulating hormone

Semen parameters of rabbit buck fed different levels of *Rubia cordifolia* root extracts (RCE)

Semen parameters of rabbit buck fed different levels of *Rubia cordifolia* root extracts (RCE) is presented in Table 5. The semen colour was milky across the treatment while sperm volume, semen pH, sperm concentration, live sperm percentage, abnormal sperm percentage and motility percentage ranged from 0.51 – 0.66 mL, 7.00 – 7.18, 21.60 – 32.34 ($\times 10^6$ /mL), 75.12 – 84.12 %, 10.04 – 14.21 % and 54.18 – 70.40 % respectively. Semen pH and colour were not significantly ($P>0.05$) different among the treatments. Conversely,

sperm volume, sperm concentration, live sperm percentage, abnormal sperm percentage and motility percentage were significantly ($P<0.05$) influenced by the treatment. According to Abd-Azim and El-kamash (2015) variation in semen colour, semen pH, semen density and motility could be attributed differences in breed of rabbit bucks. Daader and Saleem (2005); El-Sheikh and Saleem (2010) reported semen volume to increase with age and body weight. Sperm concentration and live sperm concentration in bucks fed T2, T3 and T4 were better ($P<0.05$) than those fed T1. This variation in values could be attributed to the antioxidant properties in RCE due to the presence of some secondary metabolites. Hoogenboezem and Swanepoel (2000) reported that semen quality and scrotal circumference are affected by factors related to underdevelopment of the testes and testicular degeneration. The frequency of abnormal sperm cells has been found to increase with factors such as extreme in temperature, malnutrition, toxins or anti-nutrients as well as activities of free radicals (Jimoh and Ewuola, 2018; Marai *et al.*, 2001). This has been observed to result in lower ejaculate volume and sperm motility, increase in the percentage of abnormal sperm and a decrease in the total live sperm especially among rabbit bucks in T1 (Rathore, 1970). Underdevelopment of the testis has been reported as one of the factors that can affect the quality of the semen (Ganaie *et al.*, 2013). However, the result observed in this experiment is in agreement with the findings of Ajuogu *et al.* (2018); Andrej *et al.* (2013) on the effect of herbal additive (Yucca) on rabbit spermatozoa characteristics.

Table 5: Semen parameters of rabbit buck fed different levels of *Rubia cordifolia* root extracts (RCE)

Parameters	T1	T2	T3	T4	SEM	LOS
Semen colour	Milky	Milky	Milky	Milky	-	-
Sperm volume (mL)	0.51 ^b	0.57 ^b	0.60 ^a	0.66 ^a	0.06	*
Semen pH	7.02	7.00	7.13	7.18	0.02	Ns
Sperm conc. ($\times 10^6$ /mL)	21.60 ^b	30.21 ^a	32.18 ^a	32.34 ^a	0.24	*
Live sperm (%)	75.12 ^b	80.91 ^a	82.50 ^a	84.12 ^a	0.51	*
Abnormal sperm (%)	14.21 ^a	11.84 ^b	10.10 ^b	10.04 ^b	0.03	*
Motility (%)	54.18 ^c	68.84 ^b	70.02 ^a	70.40 ^a	0.66	*

* Significant; Ns: non-significant; LOS: level of significant; T1: basal diet + 0 mL RCE; T2: basal diet + 20 mL RCE/day; basal diet + 40 mL RCE/day; basal diet + 60 mL RCE/day

4. CONCLUSION

It was concluded that *Rubia cordifolia* root extracts (RCE) is loaded with several secondary metabolites which allows it to perform multiple biological activities such as: anti-inflammatory, antioxidant, antifungal, antiviral, cytotoxic, hypolipidemic, immunomodulatory etc. RCE is relative cheap, available, effective, environmentally friendly and could be tolerated by rabbit bucks up to 60 mL per litre without causing any negative effect on the general health and performance of animals.

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Conflicts of interests

The authors declare that there are no conflicts of interests.

Data and materials availability

All data associated with this study are present in the paper.

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